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Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1-18 and 20-40 are pending in the application, with claims 1, 20, 21 and 38 being the independent claims. Claim 19 is sought to be cancelled without prejudice to or disclaimer of the subject matter therein. Claims 1-4, 7-18, 20-25, 28, 30 and 32-37 are amended. New claims 38-40 are sought to be added. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Restriction and Rejoinder

Applicants thank the Examiner for the rejoinder of claims 1-20 and 21-37 (Groups I and II, respectively) form the Restriction Requirement of August 25, 2003.

Abstract of the Disclosure

The Examiner has required amendment of the abstract to delete phraseology such as "means," "said" and "e.g." and has indicated that information given in the title should not be repeated. The Examiner has indicated that phrases which can be implied, such as, "the disclosure concerns," "the disclosure defined by the invention" and "the disclosure describes" etc. should be avoided.

The Abstract has been amended so as to meet the standards set forth by the examiner. That is, as amended, the Abstract contains less than 150 words, and does not include the words "means", "said" or "e.g." A replacement abstract is submitted herewith on a separate sheet in accord with 37 C.F.R. § 1.72. Withdrawal of the objection to the abstract is respectfully requested.

Objection to the Specification

The Examiner has objected to the use of trademarks which are not capitalized and which are not accompanied by the generic terminology. In addition, the Examiner has required that where generic terminology is specified, published product information be provided which is sufficient to show that the generic terminology or the generic description are inherent in the article referred by the trademarks.

The specification has been amended herein as required by the Examiner. In particular, trademarks have been placed in all-capital font, with generic terminology included parenthetically. The following is a listing of the trademark term, the generic terminology, and a publication source that correlates the trademark term to the generic terminology:

BUG BUSTER: Detergent containing Complete Protease Inhibitor Cocktail Tablets (Roche). See U.S. Patent 6,423,518.

BENZONASE: Recombinant Endonuclease. See U.S. Patent 6,703,484.

BRIG 35: Polyoxyethylene lauryl alcohol. See U.S. Patent 4,767,712.

ELUGENT: Mixture of alkylglucosides. See U.S. Patent 6,350,582.

GENAPOL C-100: Decaoxyethylene-dodecyl-ether. See U.S. Patent 6,165,500.

GENAPOL X-080: Octaethylene-glycol-isotridecylether. See U.S. Patent 6,165,500.

GENAPOL X-100: Decaethylene-isotridecyl-ether. See U.S. Patent 6,165,500.

PLURONIC F-127: Polyethylene-polypropylene-glycol. See U.S. Patent 6,165,500.

TRITON X-100: Nonaethylene-glycol-octyl-phenol-ether. See U.S. Patent 6,165,500.

TRITON X-114: Heptaethylene-glycol-octyl-phenylether. Condensation product of octylphenol with seven or eight moles of ethylene oxyde. Polyoxyalkylenated alkylphenol. See U. S. Patents 6,165,500, 6,261,460, 6,187,438, 5,641,480.

TWEEN 20: Polyoxyethylene-sorbitane-monolaurate. See U.S. Patent 6,165,500.

Tween 80: Polyoxyethylene-sorbitane-monooleate. See U.S. Patent 6,165,500.

B-PER: Lysis buffer containing mild nonionic detergent in 20 mM Tris HCl, pH 7.5. See Pierce product description, attached herewith.

ZWITTERGENT 3-14: N-tetradecyl-sulfobetaine or 3-(tetradecyldimethylammonio)-propane-sulfonate. See U.S. Patent 6,165,500.

APO-10: decylmethylphosphine oxide. See U.S. Patent 5,625,053.

APO-12: dodecyldimethylphosphine oxide. See U.S. Patent 5,625,053.

BigCHAP: N,N-bis(3-glucon-amidopropyl)cholamide. See U.S. Patent 6,165,500.

BigCHAP, Deoxy: N,N-bis(3-glucon-amidopropyl) deoxycholamide. See U.S. Patent 6,165,500.

BRIJ 35: 23-lauryl-ether. See U.S. Patent 6,165,500.

$C_{12}E_5$: Pentaethylene glycoldodecyl ether. See U.S. Patent 6,638,621.

$C_{12}E_6$: polyoxyethylene 6 lauryl ether. See U.S. Patent 6,015,716.

$C_{12}E_8$: polyoxyethylene 8 lauryl ether. See U.S. Patent 6,015,716.

$C_{12}E_9$: polyoxyethylene 9 lauryl ether. See U.S. Patent 6,015,716.

HECAMEG: 6-O-(N-heptylcarbamoyl)-methyl-alpha-D-glucopyranoside. See U.S. Patent 5,770,452.

MEGA-10: decanoyl-N-methyl-glucamide. See U.S. Patent 6,165,500.

MEGA-8: octanoyl-N-methyl-glucamide. See U.S. Patent 6,165,500.

MEGA-9: Nonanoyl-N-methyl-glucamide. See U.S. Patent 6,165,500.

NP-40: nonaethylene-glycol-octyl-phenyl-ether. See U.S. Patent 6,165,500.

BATC: 8,8-bis(4-aminophenyl) tricyclo(5,2,1,0.sup.2,6) decane. See U.S. Patent 6,054,554.

TOPPA: 1,1',2,2'-tetraoleoyl pyro phosphatidic acid. See U.S. patent 5,834,012.

CHAPS: 3-((cholamidopropyl)-dimethylammonio)-1-propane sulfonate. See U.S. Patent 6,165,500.

CHAPSO: 3-((cholamidopropyl)-dimethylammonio)-2-hydroxy-1-propane sulfonate. See U.S. Patent 6,165,500.

SEPHAROSE: Cross-linked agarose. See U.S. Patent 4,753,881.

SEPHADEX: cross-linked dextrans. See U.S. Patent 4,753,881.

SEPHACRYL: covalently-linked acryldextrans. See U.S. Patent 4,753,881.

TRISACRYL: copolymers of N-[tris(hydroxymethyl) methyl] acrylamide. See U.S. Patent 4,753,881.

TOYOPEARL: Polyvinyl alcohol gel. See U.S. Patent 4,414,336.

BIO-GEL: activated polyacrylamide gel. See U.S. Patent 4,414,336.

Accordingly, it is respectfully submitted that this objection has been remedied and withdrawal thereof is proper.

Rejections under 35 U.S.C. § 112

Claims 1-37 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particular point out and distinctly claim the subject matter that Applicants regard as the invention. Applicants respectfully traverse this rejection.

In particular, the Examiner states:

> Claims 1-10 are incomplete in failing to recite a process step(s) wherein the protein or peptide molecules released from cellular sources. There is no showing how the proteins or peptides are released from the cellular sources. Incorporation of the subject [matter] of claim 11 into claim 1

> with the explanation of how the "matrix" is modified to permit the limitation of claim 11 is suggested.

(Office Action, page 5, lines 4-8). The claims have been amended so that it does not contain language referring to the "release of proteins or peptides from cellular sources." It is believed that the changes to the claims obviate the basis for the rejection.

The Examiner further states:

> Claims 1, 20 and 21 are indefinite in the recitation "substantially" because the term "substantially" is a relative term, which does not identify the exact value, degree, amount, or extent of the claimed subject matter. Thus, deletion of the term "substantially" would obviate this rejection.

(Office Action, page 5, lines 9-12). Claims 1, 20 and 21 have been amended herein to delete the term "substantially."

The Examiner further states that "[c]laim 7 recites the limitation 'said release' in line 1. There is insufficient antecedent basis for this limitation in claim 1 or 7." (Office Action, page 5, lines 13-14). Claim 7 has been amended so that it does not contain the term "said release." It is believed that, as amended, the terms in claim 7 have proper antecedent basis.

The Examiner states that "[i]n claim 9, the term 'comprises' is incomplete because additional subject matter not specified in claim 7 has been added rendering claim 9 lacking proper antecedent basis. Amendment of the claim to read 'further comprising' is suggested." (Office Action, page 5, lines 15-17). Claim 9 has been amended to replace "comprises" with "further comprises."

The Examiner further states that "[c]laim 12 recites the limitation 'said filter' in line 2. There is insufficient antecedent basis for this limitation in claim 1 or claim 12." (Office Action, page 5, lines 18-19). Claim 12 has been amended to replace "filter" with

"matrix." It is believed that, as amended, the terms in claim 12 have proper antecedent basis.

The Examiner also states that "[c]laims 13 and 14 are indefinite in misspelling 'chaeotropic'. It is believed to be [a] typographical error. Appropriate correction is required." (Office Action, page 5, lines 20-21). Claims 13 and 14 have been amended to correct the spelling of "chaotropic."

The Examiner states that "[c]laim 15 is indefinite for failing to recite how the protein or peptide molecules are collected. Is it by chromatographic procedure or by filtration or by dialysis or by centrifugation? Appropriate clarification is required." (Office Action, page 6, lines 1-3). Applicants respectfully disagree that claim 15 is indefinite. However, in the interest of advancing the prosecution of the present application, claim 15 has been amended to remove the cited language.

The Examiner states that "[c]laim 20 is indefinite in the recitation 'optionally' because if an ingredient, a step, or other structural element is truly optional, i.e., its presence is not necessary for attainment of the result that is an object of the invention, then recitation thereof does not belong to the claim." (Office Action, page 6, lines 4-7). Claim 20 has been amended to remove the term "optionally."

The Examiner further states that "[c]laims 21, 34 and 35 are indefinite in the recitation '...compositions capable of being used for detecting or quantifying....'. Amendment of the claims to recite '...compositions which detect or quantify...' is suggested." (Office Action, page 6, lines 8-10). Claims 21, 34 and 35 have been amended to remove the cited language.

The Examiner further states that "[c]laim 23 recites the limitation 'said tube' in line 2. There is insufficient antecedent basis for this limitation in claim 21 or claim 23." Claim 23 has been amended so that it now depends from claim 22 which provides proper antecedent basis.

Applicants wish to thank the Examiner for suggesting changes to the claims that will obviate the present rejection. Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 102

Claims 1-2, 7-8, 11-16 and 18-20 are rejected under 35 U.S.C. § 102(b) as being anticipated by Kinsella *et al.* (U.S. Patent No. 4,427,580). Applicants respectfully traverse this rejection.

Kinsella *et al.* do not disclose contacting cells with a lysis/disruption/-permeabilization composition or compound to effect lysis of the cells. See Figure 1 which refers to mechanical disruption of yeast cells and col. 3, lines 55-58, of Kinsella *et al.* Since Kinsella *et al.* do not teach all element of Applicants' claimed invention, Kinsella *et al.* does not anticipate claims 1-2, 7-8, 11-16 and 18-20. Withdrawal of the rejection is respectfully requested.

Claims 1-2, 7-9, 11-16 and 18-20 are rejected under 35 U.S.C. § 102(b) as being anticipated by Yoshimura *et al.* (U.S. Patent No. 3,923,600).

Yoshimura *et al.* do not disclose contacting cells with a lysis/disruption/-permeabilization composition or compound to effect lysis of the cells. Instead, Yoshimura *et al.* disclose a method of purifying a lytic enzyme by culturing microorganisms that produce the lytic enzyme and treating the aqueous solution containing the lytic enzyme with acid in order to precipitate unwanted proteases. See the

abstract and the examples. At no point do Yoshimura *et al.* lyse the cells. Since Yoshimura *et al.* do not teach all element of Applicants' claimed invention, Yoshimura *et al.* do not anticipate claims 1-2, 7-9, 11-16 and 18-20. Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 103

Claims 1-37 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Kinsella *et al.* (U.S. Patent No. 4,427,580) or Yoshimura *et al.* (U.S. Patent No. 3,923,600) taken with Yoshioka *et al.* (U.S. Patent No. 4,347,316) or Henco *et al.* (U.S. Patent No. 5,652,141) or Shah *et al.* (U.S. Patent No. 4,303,530). Applicants respectfully traverse this rejection.

As discussed above, neither Kinsella *et al.* nor Yoshimura *et al.* teach or suggest contacting cells with a lysis/disruption/-permeabilization composition or compound to effect lysis of the cells. Therefore, Kinsella *et al.* and Yoshimura *et al.* are deficient references upon which to base a *prima facia* case of obviousness.

Neither Henco *et al.* nor Shah *et al.* cure the deficiencies of Kinsella *et al.* and Yoshimura *et al.* as neither reference teaches contacting cells with a lysis/disruption/-permeabilization composition or compound to effect lysis of the cells. Since all elements of the claims are not taught by the cited references, a *prima facie* case of obviousness has not been established. Withdrawal of the rejection is respectfully requested.

Other Matters

On page 2 of the Office Action, the Examiner states that document AS2 cited in Applicants' Information Disclosure Statement has not been considered as no place of

publication was provided. The place of publication is Washington, D.C., as indicated on the document. Having provided the place of publication, Applicants respectfully request that the Examiner indicate that he has considered this document in the next Office communication.

The Examiner also indicated that document AT2, U.S. application 09/478,456, was not considered as it has not been published or patented. Under 37 C.F.R.§1.97(b)(3), "[a]n information disclosure statement *shall* be considered by the Office if filed by the applicant . . . [b]efore the mailing of a first Office action on the merits" (emphasis added). Since document AT2 was cited in an Information Disclosure Statement filed October 14, 2003, and since the citation otherwise complies with the rules, the Examiner has no discretion to refuse to consider the document. Applicants respectfully request that the Examiner indicate that he has considered this document in the next Office communication.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Aaron L. Schwartz
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Date: July 2, 2004

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ABSTRACT

The present invention relates generally to compositions, methods and kits for use in extracting and isolating protein or peptide molecules. More specifically, the invention relates to such compositions, methods and kits that are useful in the isolation of protein or peptide molecules from cells. The compositions, methods and kits of the invention are suitable for isolating a variety of forms of protein or peptide molecules from cells. The compositions, methods and kits of the invention are particularly well-suited for rapid isolation of recombinant proteins or peptide molecules.

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Why Register?

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Returns Info

Current Order
Currently Empty
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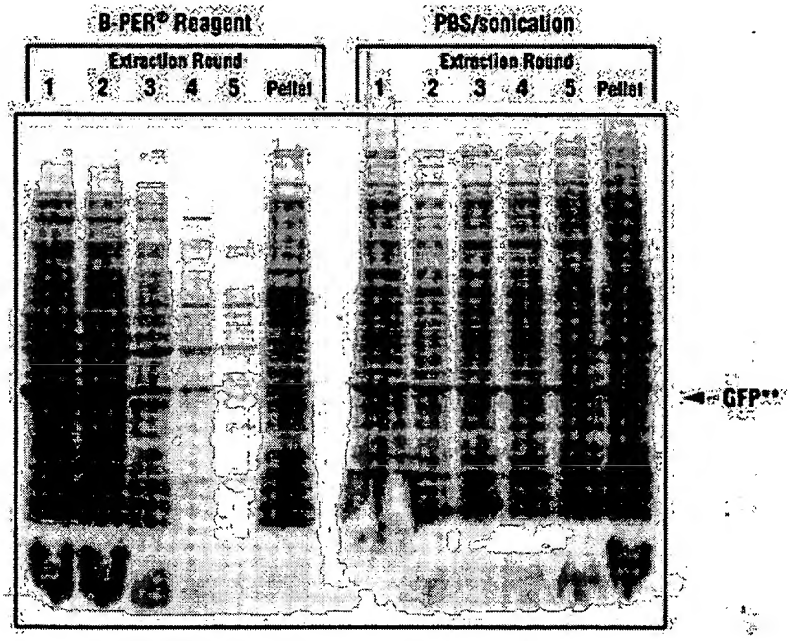
B-PER Protein Extraction Reagents

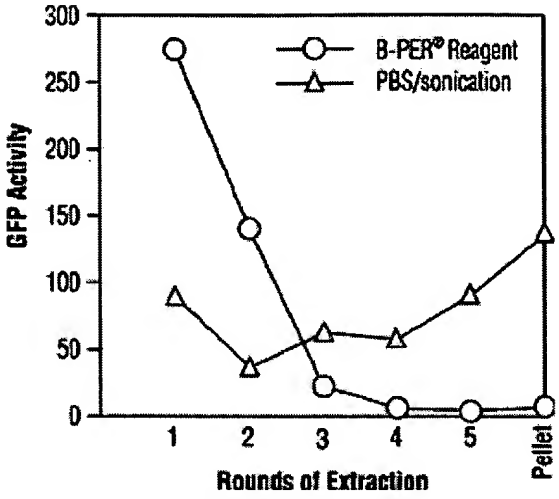
The most efficient, gentle method available for lysing bacterial *E.coli* cells

B-PER Bacterial Protein Extraction Reagent, first introduced in January 1998, provides the most efficient yet gentle method of extracting proteins, espe recombinant proteins, from bacteria (*E. coli*). This extremely easy-to-use cell ly solution utilizes a mild, nonionic detergent (proprietary) in 20 mM Tris•HCl (pH eliminating the need for mechanical cell disruption. In fact, yields obtained with PER Reagent greatly exceed those obtained using standard sonication method

How does B-PER Bacterial Protein Extraction Reagent work?
Simply add B-PER Reagent to a bacterial pellet and shake for 10 minutes. The will be disrupted and the protein of interest gently, safely released. B-PER Rea may be used for both soluble and insoluble protein extraction and inclusion bod purification from total bacterial cell lysates. Soluble proteins can be recovered b pelleting cell debris; inclusion bodies can then be purified from the pellet using optimized procedure. The novel composition of B-PER Reagent provides versa for different applications and eliminates exogenous contamination of the recom protein by the lysis reagent. Depending on the particular application, additional components such as lysozymes, protease inhibitors, salts, reducing agents, ch agents, etc. may be added to B-PER Reagent. If necessary, the nonionic deter component can be easily removed by dialysis.



* GFP = Green Fluorescent Protein
E. Coli expressing GFP was extracted five times with B-PER Reagent or PBS/sonication. Each extraction was analyzed by SDS-PAGE



E. Coli expressing GFP was extracted five times with B-PER Reagent or PBS/sonication. Each extraction was analyzed by GFP activity assay.

Features

- Easy-to-use, single solution format
- Rapid cell lysis of recombinant *E.coli* is complete in just 10 minutes
- Achieves greater yields than possible with standard sonication methods
- Flexible format that will work with any scale protein extraction
- Designed for both soluble and insoluble protein extraction
- Purifies inclusion bodies to near homogenous levels (does not solubilize inclusion bodies)

B-PER Technology is protected by U.S. Patent #6,174,704.

Ordering Information Certificate of Analysis Instruction Book

Buy	Product #	Description	Pkg. Size	Files	Pr
Add	78243	B-PER Bacterial Protein Extraction Reagent	165 ml		$
Add	78248	B-PER Bacterial Protein Extraction Reagent	500 ml		$
Add	78266	B-PER Reagent (in Phosphate Buffer)	500 ml		$
Add	89833	Lysozyme	5 g		
Add	89834	Lysozyme	25 g		$

Related Items

Downloadable Files
Recombinant Protein Extraction from *E.Coli*

Other

B-PER Bacterial Protein Extraction Reagent

Are B-PER Reagent components compatible with commercially available 6xHis- and tagged fusion protein purification columns?
B-PER Reagent is designed to be used with, among other applications, 6xHis and GST fu protein purification. Pierce sells a B-PER 6xHis Fusion Protein Purification Kit (Product # 7 B-PER 6xHis Spin Purification Kit (Product # 78300), a B-PER GST Fusion Protein Purific (Product # 78200) and a B-PER GST Spin Purification Kit (Product # 78400) specifically fc purpose.

When using B-PER Reagent with a competitor's columns, we recommend a dilution of at l to-one B-PER Reagent in the binding buffer recommended for that column. This will decre possibility of incompatibility of buffer components.

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Do cells need to be sonicated and/or frozen before treatment with B-PER Reagent?
B-PER Reagent is designed to replace sonication, which cannot fully recover soluble prote regain inclusion bodies. Although freezing the bacterial pellets is not usually necessary, it to increase protein yield in some bacteria that are traditionally difficult to lyse.

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How can I measure the amount of protein I have extracted?
The Pierce BCA Assay (Product # 23235) works very well to detect proteins extracted with Reagent.

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How much B-PER Reagent should I use for large-scale preps?
Using 1liter of Escherichia coli culture with an OD600 ~2 will give ~8 gm wet cells. For ~8 use 40-50 ml of B-PER Reagent.

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How will I know if my recombinant protein is soluble or insoluble?
PAGE examination of the protein pellet, as well as the lysate, will reveal whether the prote interest is present in inclusion bodies or if it is soluble.

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My protein is insoluble. Can I purify it using B-PER Reagent?
Inclusion bodies are easily purified using B-PER Reagent. To recover your protein from in bodies, however, we recommend the use of Pierce Inclusion Body Solubilization Reagent # 78115).

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What is the composition of B-PER Reagent?
B-PER Reagent utilizes a proprietary, mild nonionic detergent in a 20 mM Tris HCl, pH 7.5 No enzymatic components are present.

Depending on your particular protein, you may need to add components such as salt, lyso protease inhibitors, reducing agents, chelating agents, etc.

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What is the maximum amount of NaCl that I can add to B-PER Reagent for purificati salt-dependent protein?
You can add up to a final concentration of 0.5 M salt.

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Where is the DNA found when extracting proteins with B-PER Reagent?
It is found in the pellet after the first round of extraction of soluble proteins, so it should not present in the final protein preparation.

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Why is my protein extract so viscous?
This is often an indication of the presence of large amounts of DNA in the extract. This vis be greatly decreased upon addition of DNase.

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Will my protein be in its native conformation after B-PER Reagent extraction?
While this is protein-dependent, many proteins have been successfully tested in downstre applications (reporter assays, immunoprecipitation and ß-gal assays), including GST and proteins. Otherwise, samples can be diluted or dialyzed to remove any interfering substan

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